theory, employing reasonable values for the parameters, are good when relative rates are considered. In the case of the synthetic mineral rates, the relative pH and buffer concentration dependences of the $D$ and $E$ samples are similar to those for the $A$, $B$, and $C$ samples, despite differences in absolute rates. The use of $K_{HAP} = 1 \times 10^{-113}$ appears to give somewhat better agreement[8] of the synthetic mineral data with theory, but the reverse is true in the case of enamel powder. The enamel data agree remarkably well with the theory employing those same constants that gave good agreement between theory and Gray's data (3).

Continuing comparative studies include the effects of ionic strength, the effects of common ions and foreign reactive ions, such as fluoride, and a more detailed examination of the dissolution behavior near saturation. It is believed that these investigations will lead to a clear understanding of the mechanisms involved in the acid attack of enamel and in enamel reactions in general.

[8] It is noteworthy that recent (13) solubility studies with well characterized hydroxyapatite samples indicate that the $K_{sp}$ may be of this order of magnitude or even greater.

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# Percutaneous Corticosteroid Absorption Correlated to Partition Coefficient

By M. KATZ and Z. I. SHAIKH

**The vasoconstriction produced in human skin by topical corticosteroids was utilized by McKenzie and Stoughton as a biological method for measuring the efficiency of percutaneous absorption. Correlation with experimental results in this report seems to indicate that the efficiency of percutaneous absorption may be a function of physical constants, such as the product of the partition coefficient and the square root of the aqueous solubility. The results are in agreement with theoretical considerations developed by Higuchi. This suggests that increases in topical corticosteroid anti-inflammatory activity, produced by molecular modifications, are in great measure proportional to changes in solubility and partition coefficient.**

IT APPEARS THAT the water–lipid partition coefficient, originally postulated in the Meyer-Overton theory, is actually important for the absorption of substances through the skin. Those substances which combine lipoid solubility with a moderate aqueous solubility are soluble in the sebum, readily penetrate into the skin, then dissolve in the tissue fluids (1).

Valette (2) found that the cutaneous penetration of hydrocarbons, alcohols, and esters was related to liposolubility and viscosity and could be closely approximated by the rate of travel on porous porcelain impregnated with fatty acids. Treherne (3) showed that the permeability of excised skin for several radiotagged substances paralleled their ether/water partition coefficients. Stoughton and Clendenning (4) found a correlation between the penetration of the epidermal barrier by a series of nicotinic acid derivatives and their ether/water partition coefficients. They also found a similar correlation between the benzene/water partition coefficients and the penetration of the epidermis by a series of closely related boronic acid derivatives (5). The magnitude of the effect of moisture on the percutaneous diffusional rates of several salicylate esters has been shown by Wurster and Kramer (6) to be proportional to the oil–water distribution coefficient and the water solubility of these closely related compounds.

McKenzie and Stoughton (7, 8) recently have devised a technique for utilizing human skin vasoconstriction as an index of the percutaneous absorption of steroids. Since the most powerful vasoconstrictors in their series were those steroids which have been shown to be the most effective topical anti-inflammatory agents, their vasoconstriction index also might be considered an index of relative potency. The precise ranking and groupings they obtained with their *in vivo* technique suggested that the relative activity of the steroids might be correlated with *in vitro*

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properties, such as solubility or partition coefficient.

## EXPERIMENTAL

**Development of Method.**—According to the simple distribution law of Nernst, when a substance is added to a mixture of two immiscible liquids in an amount insufficient to saturate the solutions, it will become distributed between the two layers in a definite concentration ratio known as the distribution ratio, distribution coefficient, or partition coefficient (9). Reese (10) has shown that the partition coefficient is the same whether partition occurs from water into the organic phase or vice versa, and that the relative partition coefficients are the same with different solvents if no association occurs.

Since steroids are only slightly soluble in water and extremely soluble in organic liquids, it was decided that the use of a saturated aqueous solution as the phase to be partitioned would ensure low concentrations in both phases for the valid determination of partitition coefficient.

In testing possible organic phases, fixed oils and oleyl alcohol had to be abandoned because the steroid acetonides produced emulsions during the partitioning step, making separation extremely difficult. As far as determined, the oil/water partition coefficients were in the same order as the ether/water partition coefficients reported here. The high solubility of the steroids in chloroform, petroleum ether, and ether resulted in almost complete extraction from the aqueous phase, obscuring any significant differences in partition coefficient. Some improvement could be obtained by reducing the ether/water ratios from 1:1 to 1:5.

Finally, it was found that 1:1 ratios of ether and water could be used if the phases were saturated mutually with each other. This provided a highly reproducible, easily handled system for determination of partition coefficients.

**Preparation of Phases (We) and (Ew) and Partitioning.**—Distilled water and ether were mixed and allowed to stand 24 hr. The two phases then were separated into a water phase saturated with ether (We) and an ether phase saturated with water (Ew). An excess amount of steroid was added to the (We) phase in a closed bottle, which was placed in a rotating bottle apparatus (11) at 25° for 24 hr. to achieve equilibrium. The solutions then were filtered through Whatman No. 42 filter paper. This initial (We) solution was used to determine the solubility of the steroid.

Then the steroids were partitioned by shaking 100 ml. of the initial (We) solution with 100 ml. of the (Ew) phase for 20 min. The two phases were separated to produce an extracted (We) phase and an (Ew) extract.

**Method of Assay.**—The initial (We) solution and the extracted (We) phase each were extracted quantitatively with 100 ml. of chloroform which was filtered through anhydrous sodium sulfate into evaporating flasks. The (Ew) extracts also were placed in evaporating flasks. All three were evaporated carefully to complete dryness on a steam bath at a low temperature under a stream of nitrogen.

The three residues, initial (We), extracted (We), and (Ew) extract, were dissolved in methanol to a dilution suitable for the determination of absorbance in the Beckman DB spectrophotometer. Complete spectra were obtained and used for qualitative and quantitative determinations compared with spectra obtained from methanolic reference solutions of the steroids.

## RESULTS

McKenzie and Stoughton (7, 8) prepared dilutions of the corticosteroids in tenfold dilutions ranging from 1:100 to 1:10,000,000; 0.02 ml. of these dilutions were applied to 1-in. areas of the forearms and covered with Saran wrap.[1] The occluded areas were left undisturbed for 16 hr., and the number of subjects exhibiting vasoconstriction were noted for each dilution of each corticosteroid. Further vasoconstriction studies of the corticosteroid acetonides were carried out, and these observations were combined with McKenzie and Stoughton's original results. From these data, the method of least squares was used to derive a best-fit dose-response equation for each corticosteroid. This facilitated the calculation of the dilution for each corticosteroid which theoretically would produce vasoconstriction in 50% of the subjects by the McKenzie-Stoughton assay. For convenience, these values are reported as their negative logarithms in Table I under the symbol

$p$ McK-$S_{50}$ = the negative logarithm of the dilution producing vasoconstriction in 50% of the subjects by the McKenzie-Stoughton assay (Eq. 1)

The aqueous phase solubility of each steroid is listed under initial (We) in milligrams per liter. This value is converted to the square root of molar solubility under $(C_s)^{1/2}$. The concentration remaining after partitioning with the ether phase is listed under extracted (We) in milligrams per liter.

Partition coefficient (12) is described as

$$\text{partition coefficient} = \frac{\text{concn. in organic phase}}{\text{concn. in aqueous phase}} \quad \text{(Eq. 2)}$$

at equilibrium, using dilute solutions.

Since the difference between initial (We) and extracted (We) may be considered as the amount of steroid which has partitioned into the organic phase, the listed partition coefficients ($PC$) were calculated by

$$PC = \frac{\text{initial (We)} - \text{extracted (We)}}{\text{extracted (We)}} \quad \text{(Eq. 3)}$$

Assays of the (Ew) extract confirmed the values obtained by subtracting extracted (We) from initial (We).

A comparison of the McKenzie-Stoughton activity indices of the last column with the aqueous phase solubilities, expressed either as initial (We) or as $(C_s)^{1/2}$, fails to reveal any correlation. A comparison of the McKenzie-Stoughton activity indices with the $PC$ indicates some relationships.

Multiplying these two factors, the square root of the molar solubility and the partition coefficient, produces a value $[(C_s)^{1/2} \times PC]$ in Table I, which seems to correlate with the $p$ McK-$S_{50}$ values of the

[1] Dow Chemical Co., Midland, Mich.

TABLE I.—CORRELATION OF SOLUBILITY AND PARTITION COEFFICIENT TO PERCUTANEOUS ABSORPTION

| Corticosteroid | Initial (We) Concn., mg./L. | $M$ Sol. $\times 10^3$ $(C_s)^{1/2}$ | Extracted (We) Concn., mg./L. | $PC$[a] | $(C_s)^{1/2} \times PC$ | $p$ McK-$S_{50}$[b] |
|---|---|---|---|---|---|---|
| Prednisolone | 497 | 37.1 | 244 | 1.0 | 37 | 3.5 |
| 9α-Fluorohydrocortisone | 111 | 17.0 | 34 | 2.3 | 39 | 3.6 |
| Methylprednisolone | 129 | 18.5 | 36 | 2.6 | 48 | 3.2 |
| Hydrocortisone | 585 | 40.1 | 258 | 1.3 | 52 | 3.5 |
| Hydrocortisone acetate | 14 | 5.9 | 1 | 13 | 77 | 4.2 |
| Prednisolone acetate | 28 | 8.3 | 2 | 13 | 108 | 4.4 |
| Dexamethasone | 121 | 17.6 | 16 | 6.6 | 116 | 4.7 |
| 9α-Fluorohydrocortisone acetate | 54 | 11.3 | 3 | 17 | 192 | 4.6 |
| Triamcinolone acetonide | 41 | 9.7 | 3 | 12.7 | 123 | 5.4 |
| Fluocinolone acetonide | 108 | 15.4 | 6 | 17 | 262 | 5.7 |
| Flurandrenolone (acetonide) | 295 | 26.0 | 22 | 12.4 | 322 | 5.6 |

[a] $PC$ (partition coefficient) = (initial (We) concn. − extracted (We) concn.)/extracted (We) concn. [b] Negative logarithm of dilution producing vasoconstriction in 50% of subjects adapted from McKenzie, A. W., and Stoughton, R. B., *A.M.A. Arch. Dermatol.*, **86**, 608(1962).

steroids. The relationship is depicted in Fig. 1 along with the calculated regression line. The correlation coefficient is 0.86 at $P = < 0.01$.

## DISCUSSION

Walton (13) found that the sublingual absorption of alkaloids, organic nitrates, and steroids was a highly selective process and that the most important determining factor was the oil–water partition coefficient. It was his belief that the same conditions of solvent competition prevail between the aqueous salivary fluids and the fat in the cell protoplasm, as in the experimental oil–water partitioning system. He found that the penetrability of drugs was so conditioned by this physicochemical factor that the penetrability could be predicted with fair accuracy within a group of chemically related drugs. For alkaloids, a partition coefficient of 20–30 was minimal, and 40–2000 was optimum for sublingual penetration. Alkaloids with coefficients over 2000 were too oil soluble and too water insoluble to obtain a sufficient concentration in the aqueous salivary fluids for presentation to the sublingual absorption site. In essence, the aqueous solubility of a drug determines the concentration presented to the absorption site, and the partition coefficient strongly influences the rate of transport across the absorption site.

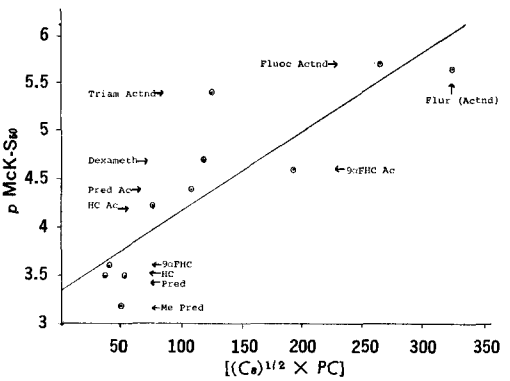


Fig. 1.—Correlation of the dilution of topical corticosteroids which produces vasoconstriction in 50% of the subjects by the McKenzie-Stoughton assay ($p$ McK-$S_{50}$) with the product of their solubility and partition coefficient $[(C_s)^{1/2} \times PC]$.

Some of the processes involved in securing therapeutic effect when topical preparations containing dispersed drug particles are applied to the skin are (14): (*a*) solution of drug in vehicle, (*b*) diffusion of drug through vehicle, (*c*) transfer of drug from vehicle to skin, and (*d*) diffusion of drug through skin.

The first two factors have been subjected to a quantitative treatment by Higuchi (15), who showed that the amount of drug released from a suspension-type ointment ($C_s \ll A$) is not directly proportional to concentration but is proportional to the square roots of the amount of drug per unit volume, drug solubility in vehicle, diffusion constant in vehicle, and time.

$$\frac{dQ}{dt} = \sqrt{\frac{ADC_s}{2t}} \qquad \text{(Eq. 4)}$$

where

$Q$ = the amount absorbed at time $t$ per unit area of exposure.
A = the concentration of drug expressed in unit/cm.$^3$.
$C_s$ = the solubility of the drug as units/cm.$^3$ in the external phase of the ointment.
$D$ = the diffusion constant of the drug molecule in the external phase.

Higuchi (15) also developed a permeability constant which quantifies the third and fourth factors as the product of the drugs $PC$ and its diffusivity ($Db$) in the skin:

$$\text{permeability constant} = (PC) \times (Db) \qquad \text{(Eq. 5)}$$

Therefore, it is proposed that the rate of percutaneous absorption of drugs applied as fine suspensions can be described as a function of the product of the amount of drug released from the vehicle to the absorption site and its permeability constant for transport through the barrier phase. Combining the two equations of Higuchi (15) yields

$$\frac{dQ}{dt} = \sqrt{\frac{ADC_s}{2t}} \times (PC) \times (Db) \qquad \text{(Eq. 6)}$$

In the comparison of steroid molecules of similar shapes and molecular weights, differences in diffusivity, ($D$) and ($Db$), can be disregarded readily since it only varies inversely with the cube root of the molecular weight (Stokes-Einstein equation).

Since we are interested in the relative percutaneous absorption of corticosteroids, theoretical condi-

tions for comparison would presume equal amounts ($A$) of steroids in identical vehicles applied for equal periods of time ($t$), canceling out these two parameters. Therefore, the above equations can be simplified for our purposes to

$$\frac{dQ}{dt} = K\sqrt{C_s} \times PC \qquad \text{(Eq. 7)}$$

The relative rates of percutaneous absorption for similar substances (in equal concentrations in identical vehicles during equal time periods) may thus be described as a function of the product of ($a$) the amount of drug released to the absorption site from the vehicle (which is proportional to the square root of the drug solubility in the external phase of the vehicle) and ($b$) the permeability through the skin (which is proportional to the partition coefficient).

The values $[(C_s)^{1/2} \times PC]$ calculated from the experimental data for each corticosteroid are presented in Table I. A direct correlation is suggested when these values for each corticosteroid are compared graphically with their $p$ McK-$S_{50}$ values in Fig. 1.

The McKenzie-Stoughton activity index of human skin vasoconstriction may be considered a biological method for measuring the efficiency of percutaneous absorption. The correlation with the experimental results reported here indicates that the efficiency of percutaneous absorption may be a function of the physical constants of solubility and partition coefficient. This suggests that increases in topical corticosteroid anti-inflammatory activity produced by molecular modifications are in great measure proportional to changes in solubility and partition coefficients.

Examination of the physical properties of the members of each group reveals interesting similarities. The group of compounds which produces vasoconstriction at a maximum dilution of $10^{-3}$ ($p$ McK-$S_{50}$ of 3–4) consists of hydrocortisone and three closely related compounds, prednisolone, 6$\alpha$-methylprednisolone, and 9$\alpha$-fluorohydrocortisone. These compounds show a strong similarity of physical properties, relatively high solubilities with a $(C_s)^{1/2}$ of 17 to 40, and low partition coefficients of 1 to 2.6, resulting in $[(C_s)^{1/2} \times PC]$ values ranging from 37 to 52.

Dexamethasone, which primarily differs from the members of the previous group by having a 16$\alpha$-methyl group, exhibits a similar solubility but an increase of partition coefficient to 6.6, resulting in a $(C_s)^{1/2} \times PC$ value of 116, and the capability of producing vasoconstriction at a tenfold increased dilution of $10^{-4}$ ($p$ McK-$S_{50}$ of 4–5). The other members of this $10^{-4}$ group are the acetates of the compounds in the $10^{-3}$ group. These acetates are much less soluble than their parent compounds, with $(C_s)^{1/2}$ ranging from 6 to 17 but have higher partition coefficients ranging from 13 to 17, resulting in $(C_s)^{1/2} \times PC$ values of 77 to 192. It is interesting to speculate that higher esters may result in slightly greater partition coefficients but lower aqueous phase solubilities.

The three compounds which exhibit vasoconstriction at the low dilutions of $10^{-5}$ ($p$ McK-$S_{50}$ of 5–6) are all 16$\alpha$-hydroxy, 16$\alpha$,17$\alpha$-acetonides. The acetonides' partition coefficients, ranging from 12 to 17, are similar to those of the acetates of the $10^{-4}$ group. However, the aqueous phase solubilities of flurandrenolone (acetonide) and fluocinolone acetonide are significantly higher, resulting in $(C_s)^{1/2} \times PC$ values of 322 and 262, respectively. Triamcinolone acetonide seems to be an exception since it possesses a relatively lower solubility.

## CONCLUSIONS

A correlation between the relative percutaneous absorption of topical corticosteroids and the simple physical properties of solubility and partition coefficient has been demonstrated. These factors by themselves are, of course, insufficient to explain completely anti-inflammatory activity.

Compounds with suitable physical properties for percutaneous absorption but without suitable biological properties for anti-inflammatory activity cannot be effective and vice versa. After achieving absorption, biological events, such as plasma protein binding (16), receptor specificity, and rate of metabolic transformation or inactivation (17) certainly play a role in accomplishing topical anti-inflammatory activity. Studies of the relative biological properties of the compounds discussed in this paper are in progress.

It might be concluded that the structural changes which produced potent topical anti-inflammatory corticosteroids provided the physical properties necessary first to achieve effective percutaneous absorption, then effective biological activity.

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